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Original Research Article

Association of serum CXCL-10, IL-22 and IL-6 with types of vitiligo - Study conducted at a tertiary care hospital of Tripura

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Abstract

Background: According to research conducted in India, the prevalence of vitiligo in dermatology outpatients consistently ranges from 0.25 to 4 percent. From previous studies, it has been reported that the serum level of CXCL-10 is increased in organ specific autoimmune diseases, such as type 1 diabetes, Graves' disease etc. But the role of CXCL10 in the immunopathogenesis of vitiligo has been highlighted in very few studies.
Objectives: To estimate the serum levels of CXCl-10, IL-6 & IL-22 in patients suffering from Vitiligo reporting in Dermatology Outpatient Department and also to find out the association between these serum biomarkers and clinical status of Vitiligo among the study subjects.
Patients and Methods: The study included One hundred forty-two vitiligo patients who were able to comply with the study protocol reported in the Dermatology Outpatient department of a tertiary teaching hospital of Tripura. All recruited patients were subjected to documentation of complete history. Detailed physical examination was performed for type of vitiligo, the distribution and body surface area (BSA) involvement using palm method and rule of nines. 5 ml Blood Samples were taken after getting written consent from the patient or from the legal guardian in case of children for performing tests namely Serum CxCL-10, IL-6, IL-22 etc. Results: Out of 142 vitiligo patients, 66.2% (94) were Female and 33.8% (48) were Male. According to study result maximum Vitiligo patients of Tripura lie within 11-20 years age group (24.65%). The mean serum levels of CXCL10 and IL-22 in the Vitiligo group was statistically higher in patients with Non-segmental type of Vitiligo.
Conclusion: From this study, we can conclude that increased serum levels of CXCL10 is associated with types of Vitiligo where non-segmental type showed maximum level followed by segmental and focal type respectively.

Keywords: Vitiligo, Tripura, CxCL-10, Non-segmental vitiligo

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1. Introduction

Geographical locations and sample sizes have an impact on the prevalence of vitiligo. With prevalence estimates ranging from 0.1% to 2.0% based on general populations in prior studies, this condition also affects a variety of ethnic groups. One can be affected by vitiligo at any age and normally it affects both the sexes.[1] Type 1 diabetes, Graves' disease, and other organ-specific autoimmune disorders have been shown to have elevated serum levels of CXCL-10.[2,3] However, only a small number of studies have emphasized that CXCL10 plays in the immunopathogenesis of vitiligo. According to Ratsep et al., IL-22 provokes inflammation thereby resulting in melanocyte destruction; however, the actual role of serum IL-22 in Vitiligo still needs to be elucidated.[4,5] Similarly, IL-6 is involved in the pathogenesis of several autoimmune diseases, but its role in Vitiligo has not been well defined specially among different ethnic & non ethnic population of our country. However, only a few studies reported the relationship between CXCl-10, IL-6 and risk of Vitiligo. Very limited data is available about association of these Serum biomarkers in Vitiligo patients in India.

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Ethnic & non-ethnic people of Tripura have different life style, different food habit & different socio- economic status. Around one-third of the population of Tripura belongs to the Scheduled Tribes. Tripura's tribal population is different from the rest of the country in terms of their dietary habits, way of life, sociocultural background, and genetic composition. Nowadays, a greater number of vitiligo sufferers are visiting dermatology outpatient departments in small states like Tripura. However, as of now, no research has been done on Vitiligo in the people of Tripura. So, considering the important role of cytokines in autoimmune diseases, this study was planned to estimate the serum levels of CXCl-10, IL-6 & IL-22 in patients suffering from Vitiligo reporting in Dermatology OPD of AGMC and also to find out the association between these serum biomarkers and clinical status of Vitiligo among the study subjects.

2. Materials and Methods

This hospital based cross sectional study was conducted for 2 years i.e from March, 2021 to February, 2023 among One hundred forty two (142) Vitiligo patients attending Dermatology Outpatient Department of a Tertiary care teaching hospital of Tripura. Sample size was calculated by using the formula $n = [Z^2_\alpha \{p (1-p)\}] \div d^2$. Where: n is the sample size. Z_α is z statistic for the level of confidence and in this study $Z_\alpha = 1.96$ at 95% confidence. P is the expected proportion and here p = 9.98%, considering 9.98% of the Indian population suffer from Vitiligo[6] and d is absolute error and here d is considered as 5%. All patients diagnosed with Vitiligo fulfilling the criteria given by Sehgal et al. on Indian population were included in this study.[7] Vitiligo patients suffering from other diseases that can affect any of the measured activity markers, such as systemic autoimmune diseases (e.g. autoimmune thyroiditis, rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, sarcoidosis, allergic asthma) (by history & also by clinical tests) and dermatological diseases (psoriasis, systemic sclerosis, alopecia areata, malignant melanoma, basal cell carcinoma, lichen planus) were excluded from this study. A comprehensive history about age, sex, occupation, duration, and site of infection was obtained from each research participant. Individuals already undergoing therapy were excluded from the study. Detailed physical examination was performed for type of vitiligo, the distribution and body surface area (BSA) involvement using palm method and rule of nines. Blood samples were collected with written consent from the patient or the legal guardian in the case of minors. Simultaneously blood sample was also collected from one blood relative from each of the patient. Then 5ml venous blood samples were collected from both study subject & control for performing the following tests by maintaining standard guidelines of WHO:[8] 1. Random Blood Sugar (FBS), HbA1c, Lipid Profile by Autoanalyser (XL-640). 2. Estimation of anti-thyroid peroxidase (Anti-TPO) and thyroid stimulating hormone (TSH) by using enzyme-linked immunosorbent assay technique (ELISA Reader- MultisKan FC Microplate Reader) with the commercially available ELISA Kit. 3. Complete blood count (CBC) by Haematoanalyser (XN-1000). 4. Measurement of serum CXCL-10, IL-6 & IL-22 by ELISA (enzyme-linked immunosorbent assay) method using a commercial ELISA kit. Data management: At first data (Including lab report) was recorded in the interview schedule and later on entered and analyzed in computer using SPSS 25 for windows. Data will be presented using text, tables and charts. Statistical analysis: Data was represented as mean ± standard deviation (SEM). The studied variables were compared using Student's t-test and the strength of linear correlation was assessed with Pearson's Correlation coefficient (r). The Chi-square test was employed for the comparison of categorical data. Data were consideration statistically significant at a p value of less than 0.05.

3. Results

This study was conducted among One hundred and Forty two Vitiligo patients and one blood relative from each of the patient. The overall institutional prevalence of vitiligo was found to be 0.77% (142 out of 18348). Out of 142 vitiligo patients, 66.2% (94) were Female and 33.8% (48) were Male. In this study, the age range of the vitiligo patients were 9-65 years with a mean of 36.59 ± 17.4 years (Median - 38.50 years). 89% of the studied group had a negative family history of vitiligo. Out of 142 vitiligo patients all the study subjects were non-ethnic.

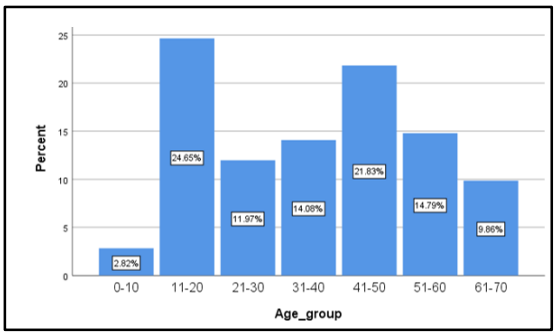

Figure 1: Age group distribution

Figure 1 depicted the age group distribution among the study subjects where it showed that maximum Vitiligo patients of Tripura lie within 11-20 years age group (24.65%). Most cases (*n* =112, 78.8%, excluding Segmental Vitiligo and Mucosal Vitiligo cases) had less than 10% body surface area (BSA) involvement. Neck region was the most commonly affected initial site of involvement (*n* = 142, 21.3%) across all clinical types. However, it was also the most commonly affected region among non-segmental cases (**Figure 2**).

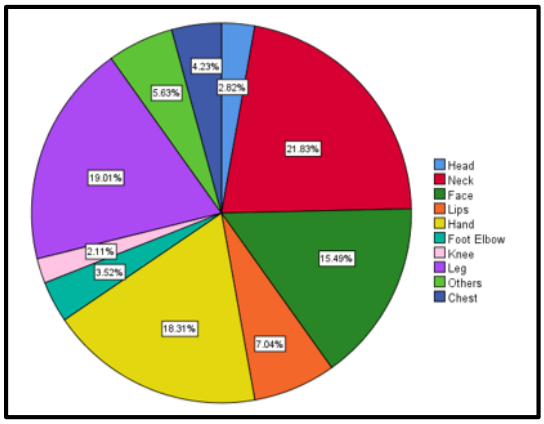

Figure 2: Frequency of initial site of involvement

Among 142 vitiligo patients 90.1% are having non-segmental type of Vitiligo which may be seen at **Table 1**. Depicts the non-segmental type of vitiligo patient include in our study. Out of 142 cases 114(80.3%) cases are stable for more than 3 months whereas 19.7 % cases are actively spreading since the last month. The values of CxCL10 and IL-6 were significantly higher in the vitiligo group as compared to the controls ($p < 0.05$), whereas no association was found in serum levels of IL-22 among the two groups.

The mean serum level of CXCL10 in the vitiligo group was statistically higher than the mean serum level of CXCL10 in the blood relative group (468.3±107.1 pg/mL vs. 384.5± 116.2 pg/mL, respectively) [**Table 2**]. Similarly, the mean serum level of IL-6 in the studied vitiligo group was statistically higher than that of the mean serum level of IL-6 in the other group (43.3±32.5 ng/mL vs. 39.4±29.4ng/mL). In our study no significant difference was found between mean serum levels of lipid profile parameters among the two groups.

Regarding vitiligo type, the mean serum level of CXCL10 in the studied vitiligo group was statistically higher in patients with Non-segmental vitiligo than among either patients with segmental type or Focal Type (475.03 ± 106.9 pg/mL vs. 447.5±103.4 and 386.6 ± 82.0 respectively) [**Table 3**]. Similarly the mean serum level of IL-22 in the studied vitiligo group was statistically higher in patients with non-segmental vitiligo than those with other types of vitiligo (4.02±1.02 ng/mL vs. 3.58±1.05 and 2.36±0.18 ng/mL respectively). **Table 4** represents association between types of vitiligo and nature of vitiligo where it showed that out of 128 non-segmental type of vitiligo 101 are of progressive nature.

Table 1: Frequency percentage of types of Vitiligo

Type of Vitiligo	Frequency (Percentage)
Segmental	4 (2.8%)
Non Segmental	128(90.1%)
Focal	9(6.3%)
Mucosal	1(0.7%)
Total	142

Table 2: Comparison of parameters among the two groups

Parameters	Vitiligo patients (Mean ± SD)	Blood relative (Mean ± SD)	p-value*
Hemoglobin levels (g/dL)	12.1± 1.40	12.2± 1.45	0.554
IL-22	34.7 ± 11.9	30.5± 29.1	0.117
CXCL 10	468.3± 107.1	384.5± 116.2	0.001
IL-6	43.3 ± 32.5	39.4 ± 29.2	0.004
Cholesterol	128.8 ± 21.7	132.2 ± 28.4	0.257
Triglyceride	123.02 ± 22.6	128.4± 29.4	0.085
HDL	41.5± 10.2	41.6± 10.9	0.910
LDL	58.09± 12.5	60.7± 19.2	0.166

Table 3: Association of types of vitiligo and serum levels of parameters

Parameters	Vitiligo Type	Mean ± SD	P value
IL-22	Non-segmental	34.7± 12.1	0.725
	Focal	33.3± 7.0	
	Segmental	32.5±17.5	
CxCL-10	Non-segmental	475.03 ± 106.9	0.017
	Focal	386.6 ± 82.0	
	Segmental	447.5±103.4	

IL-6	Non-segmental	44.4 ± 30.3	0.011
	Focal	34.4 ± 7.7	
	Segmental	26.2±13.7	

Table 4: Types of vitiligo Vs Nature of vitiligo

Nature of Vitiligo	Types of Vitiligo				Pearson Chi square (P Value)
	Segmental	Non-Segmental	Focal	Mucosal	286.54 (<0.001)
Progressive	2 (1.4%)	101 (71.1%)	5 (3.5%)	0	
Stable	2 (1.4%)	27 (19.0%)	4 (2.8%)	1 (0.70%)	
Total	4	128	9	1	

4. Discussion

This study included One hundred and forty two Vitiligo patients attending Dermatology outpatient department of Agartala Government Medical College and GBP hospital. Female gender predominated in this present study. This is because early consultation is sought by women due to marital worries and social stigma. According to some studies vitiligo was more common in males whereas others have found it to be more common in females.[9-14] Even some have found no difference in both genders.[15-17] So, it is important to learn how this complex disease manifests differently in men and women so that we can identify the unique genetic elements that may contribute to its development. In this study, 24.65% of the patients, the age at onset of disease was 11-20 years, which is consistent with most studies from India. This implied that the disease starts at a younger age in the Indian population. Although many studies have revealed that prevalence of vitiligo varies on ethnicity,[18] present study did not find a single ethnic vitiligo study participant from this tertiary care center within this study period. Nonsegmental vitiligo has a worse prognosis than segmental vitiligo since our study found that it progresses at a significantly higher rate.

The present study revealed that serum CXCL10 levels were significantly higher in vitiligo patients than in controls (p=0.001). The findings correspond with the study by Ferrari et al., which examined serum CXCL10 in non-segmental vitiligo, demonstrating a statistically significant difference in serum CXCL10 levels between patients and controls (p=0.0001), with increased levels noted in vitiligo patients. In this study, patients with vitiligo exhibited elevated levels of CXCL10 in their blood samples, as highlighted by Rashighi et al.[19] This aligns with the findings of Wang et al.,[20] which indicated that CXCL10 levels were considerably elevated in patients with vitiligo. CXCL10 is known to have a significant impact on T cell activation and tethering.[21] So, CxCL-10 may facilitate the location of autoreactive T cells to the epidermis, hence initiating vitiligo which was already observed in mouse model in a previous study.[19]

Our investigation found that IL-6 serum concentrations were much higher in the patients compared to the controls. Research on vitiligo instances by multiple researchers could provide credence to these findings.[22] The production of Il-6 by mononuclear cells has the potential to stimulate the expression of intercellular cell adhesion molecules (ICAM-1) on melanocytes, potentially enhancing leukocyte-melanocyte interactions. This interaction may result in polyclonal B-cell activation and an increase in autoantibody production, ultimately causing immunological damage to the melanocytes. Singh *et al.*[23] also reported significantly increased IL-6 in vitiligo patients than controls. Many other researchers also have reported high IL-6 levels in vitiligo patients.[24,25]

Ethical Clearance: The ethical permission was taken before commencement of the study from the Institutional Ethics Committee (Ref no. F.4(5-234)/AGMC/Academic/IECMeeting/2020/783, Dated, 18/01/2021). The data generated during this study was handled with confidentiality and utilized solely for research purposes.

5. Limitations

Short span of observation was one of the limitations of this study. Other than this, the design of this study was hospital-based which might not reflect the accurate situation of vitiligo in this region.

6. Conclusion

This study concludes that the types of vitiligo correlates with elevated serum levels of CXCL10. The inhibition of CXCL10 and IL-22 activities will offer novel therapeutic avenues for vitiligo treatment.

7. Ethical Approval

This study was ethically approved by institutional ethical committee with ref. no. F.4(5-234)/AGMC/Academic/IEC Meeting/2020/3151.

8. Conflict of Interest

None.

9. Source of Funding

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